

§Appl. No. 10/009,500
Amdt. dated August 23, 2004
Reply to Office Action of, April 21, 2004

Listing of Claims:

Please amend the claims as follows:

Claim 1 (Original) A purified protein isolated from the leech species *Hirudinaria manillensis* having the biological activity of a hyaluronidase which is not influenced in its activity by heparin, characterized in that it has a molecular weight of 53 – 60 dependent on glycosylation.

Claim 2 (Original) A glycosylated protein according to claim 1 having a molecular weight of 58 (± 2).

Claim 3 (Original) A non-glycosylated protein according to claim 1 having a molecular weight of 54 (± 2).

Claim 4 (Previously Presented) A protein according to claim 1 having an isoelectric point of 7.2 - 8.0.

Claim 5 (Previously Presented) A protein according to claim 1 having the amino acid sequence given in Fig. 7 and SEQ ID No. 1.

Claim 6 (Previously Presented) A protein according to claim 1 having a specific enzymatic activity of > 100 kU / mg protein.

Claim 7 (Currently Amended) A process for isolating and purifying the protein as defined in of claim 1 comprising: the following steps

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- (i) homogenization of heads of leeches of the species *Hirudinaria manillensis* with an acid buffer and centrifugation,
- (ii) ammonium sulfate precipitation of the supernatant of step (i),
- (iii) cation exchange chromatography,
- (iv) concanavalin A affinity chromatography,
- (v) hydrophobic interaction chromatography,
- (vi) affinity chromatography on matrices coated with hyaluronic acid fragments, and
- (vii) gel permeation chromatography,
and optionally
- (viii) enzymatic or chemical de-glycosylation of the purified protein.

Claim 8 (Original) A protein having the biological activity of a hyaluronidase which is not influenced in its activity by heparin and having a molecular weight of 53 – 60 dependent on glycosylation, obtainable by the process steps of claim 7.

Claim 9 (Original) A protein according to claim 8 having a specific enzymatic activity of > 100 kU / mg protein.

Claim 10 (Withdrawn) A DNA sequence coding for a protein of claim 1.

Claim 11 (Withdrawn) A DNA sequence coding for a protein of claim 8 comprising any nucleotide sequence depicted in Fig. 8 (SEQ. ID No. 2) , Fig. 9 (SEQ. ID No. 4) and Fig.10 (SEQ ID No. 6).

Claim 12 (Withdrawn) A recombinant protein having the biological activity of a hyaluronidase encoded by any a DNA sequence of claim 11.

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Claim 13 (Withdrawn) A recombinant protein with the biological activity of a hyaluronidase and a molecular weight of 55 – 59 dependent on glycosylation having any amino acid sequence depicted in Fig. 8, 9 and 10 (SEQ. ID Nos. 3, 5, 7) or a sequence which has a homology to said sequences of at least 80%.

Claim 14 (Withdrawn) An expression vector comprising a DNA sequence of claim 10.

Claim 15 (Withdrawn) A host cell suitable for the expression of a protein of claim 12 which was transformed with a vector comprising a DNA sequence for a protein comprising any nucleotide sequence depicted in Fig. 8 (SEQ. ID No. 2), Fig. 9 (SEQ. ID No. 4) and Fig. 10 (SEQ ID No. 6).

Claim 16 (Currently Amended) A pharmaceutical composition comprising a protein according to any of claim 1 as a medicament and a pharmaceutically acceptable diluent, carrier, or excipient therefor.

Claim 17 (Original) A pharmaceutical composition comprising the protein of claim 16 and a pharmaceutically acceptable diluent, carrier or excipient therefor.

Claim 18 (Original) A pharmaceutical composition comprising additionally a pharmacologically active compound.

Claim 19 (Original) A pharmaceutical composition according to claim 18, wherein the pharmacological active compound is heparin.

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Claim 20 (Currently Amended) ~~The use of a protein according to claim 1 in the manufacture of a medicament for A method of treating myocardial, cardiovascular and thrombotic disorders and tumors, comprising administering to a subject in need of a protein of claim 1.~~

Claim 21 (New) A process of claim 7, further comprising:

(viii) enzymatic or chemical de-glycosylation of the purified protein.

Claim 22 (New) A protein of claim 1 having the amino acid sequence of SEQ ID NO: 3.

Claim 23 (New) A protein of claim 1 having the amino acid sequence of SEQ ID NO: 5.

Claim 24 (New) A protein of claim 1 having the amino acid sequence of SEQ ID NO: 7.